

WHAT IS CLAIMED IS:

1                   1.     A *Lactobacillus* bacterium comprising an expression cassette, the  
2 expression cassette comprising a promoter operably linked to polynucleotide encoding a  
3 signal sequence and a biologically-active polypeptide, wherein the biologically active  
4 polypeptide is linked to a heterologous carboxyl terminal cell wall targeting region and  
5 wherein the heterologous carboxyl terminal cell wall targeting region comprises in the  
6 following order:

7                   a cell wall associated sequence;  
8                   LPQ(S/A/T)(G/A);and  
9                   a hydrophobic sequence.

1                   2.     The *Lactobacillus* bacterium of claim 1, wherein the cell wall  
2 associated sequence comprises at least 50 amino acids.

1                   3.     The *Lactobacillus* bacterium of claim 1, wherein the cell wall  
2 associated sequence comprises at least 200 amino acids.

1                   4.     The *Lactobacillus* bacterium of claim 1, wherein the heterologous  
2 carboxyl terminal cell wall targeting region further comprises a charged sequence at the  
3 carboxyl terminus of region.

1                   5.     The *Lactobacillus* bacterium of claim 1, wherein the *Lactobacillus*  
2 bacterium is a vagina-colonizing strain.

1                   6.     The *Lactobacillus* bacterium of claim 1, wherein the *Lactobacillus*  
2 bacterium is selected from the group consisting of *L. jensenii*, *L. gasseri*, and *L. casei*.

1                   7.     The *Lactobacillus* bacterium of claim 1, wherein the cell wall targeting  
2 region comprises the amino acid sequence LPQSG.

1                   8.     The *Lactobacillus* bacterium of claim 1, wherein the cell wall targeting  
2 region comprises the amino acid sequence LPQAG.

1                   9.     The *Lactobacillus* bacterium of claim 1, wherein the cell wall targeting  
2 region comprises the amino acid sequence LPQTG.

- 1                   10.     The *Lactobacillus* bacterium of claim 1, wherein the cell wall targeting  
2     region comprises the amino acid sequence LPQTA.
- 1                   11.     The *Lactobacillus* bacterium of claim 1, wherein the cell wall targeting  
2     region comprises SEQ ID NO:7.
- 1                   12.     The *Lactobacillus* bacterium of claim 1, wherein the cell wall targeting  
2     region comprises SEQ ID NO:8.
- 1                   13.     The *Lactobacillus* bacterium of claim 1, wherein the biologically  
2     active polypeptide is expressed in the cell wall of the bacterium.
- 1                   14.     The *Lactobacillus* bacterium of claim 1, wherein the biologically-  
2     active polypeptide is between 10 and 600 amino acids.
- 1                   15.     The *Lactobacillus* bacterium of claim 1, wherein the biologically  
2     active protein binds to a pathogen when the biologically active protein is contacted with the  
3     pathogen.
- 1                   16.     The *Lactobacillus* bacterium of claim 15, wherein the pathogen is a  
2     bacterial pathogen.
- 1                   17.     The *Lactobacillus* bacterium of claim 15, wherein the pathogen is a  
2     fungal pathogen.
- 1                   18.     The *Lactobacillus* bacterium of claim 15, wherein the pathogen is a  
2     viral pathogen.
- 1                   19.     The *Lactobacillus* bacterium of claim 18, wherein the viral pathogen is  
2     HIV.
- 1                   20.     The *Lactobacillus* bacterium of claim 19, wherein the biologically  
2     active protein is CD4 or an HIV-binding fragment of CD4.
- 1                   21.     The *Lactobacillus* bacterium of claim 19, wherein the biologically  
2     active protein is 2D-CD4.

22. The *Lactobacillus* bacterium of claim 18, wherein the biologically active protein is cyanovirin-N or a virus-binding fragment of cyanovirin-N.

23. The *Lactobacillus* bacterium of claim 18, wherein the viral pathogen is herpes simplex virus.

24. The *Lactobacillus* bacterium of claim 18, wherein the biologically active protein is herpes simplex virus entry mediator C (HveC) or a virus-binding fragment of HveC.

25. The *Lactobacillus* bacterium of claim 1, wherein the biologically active polypeptide is released from the *Lactobacillus* bacterium.

26. The *Lactobacillus* bacterium of claim 4, wherein the biologically active polypeptide is anchored to the cell wall of the *Lactobacillus* bacterium.

27. A method of expressing a biologically active polypeptide in the cell wall of a *Lactobacillus* bacterium, the method comprising providing a *Lactobacillus* bacterium comprising an expression cassette, the expression cassette comprising a promoter operably linked to a polynucleotide encoding a signal sequence and a biologically-active polypeptide, wherein the biologically active polypeptide is linked to a heterologous carboxyl terminal cell wall targeting region and wherein the heterologous carboxyl terminal cell wall targeting region comprises in the following order:

a cell wall associated sequence;

LPQ(S/A/T)(G/A);and

a hydrophobic sequence; and

culturing the bacterium under conditions to induce expression of the polypeptide, thereby expressing a biologically active polypeptide in the cell wall of a *Lactobacillus* bacterium.

28. The method of claim 27, wherein the cell wall associated sequence comprises at least 50 amino acids.

29. The method of claim 27, wherein the cell wall associated sequence comprises at least 200 amino acids.

- 1                   30.     The method of claim 27, wherein the heterologous carboxyl terminal  
2 cell wall targeting region further comprises a charged sequence at the carboxyl terminus of  
3 region.
- 1                   31.     The method of claim 27, wherein the providing step comprises  
2 transferring the expression cassette into the bacterium.
- 1                   32.     The method of claim 27, wherein the cell wall targeting region  
2 comprises the amino acid sequence LPQSG.
- 1                   33.     The method of claim 27, wherein the cell wall targeting region  
2 comprises the amino acid sequence LPQAG.
- 1                   34.     The method of claim 27, wherein the cell wall targeting region  
2 comprises the amino acid sequence LPQTG.
- 1                   35.     The method of claim 27, wherein the cell wall targeting region  
2 comprises the amino acid sequence LPQTA.
- 1                   36.     The method of claim 27, wherein the cell wall targeting region  
2 comprises SEQ ID NO:7.
- 1                   37.     The method of claim 27, wherein the cell wall targeting region  
2 comprises SEQ ID NO:8.
- 1                   38.     The method of claim 27, wherein the cell wall targeting region  
2 comprises at least 200 amino acids.
- 1                   39.     The method of claim 27, wherein the *Lactobacillus* bacterium is a  
2 vagina-colonizing strain.
- 1                   40.     The method of claim 27, wherein the *Lactobacillus* bacterium is  
2 selected from the group consisting of *L. jensenii*, *L. gasseri*, and *L. casei*.
- 1                   41.     The method of claim 27, wherein the biologically-active polypeptide is  
2 between 10 and 600 amino acids.

- 1                   42.     The method of claim 27, wherein the biologically active protein binds  
2     to a pathogen when the biologically active protein is contacted with the pathogen.
- 1                   43.     The method of claim 42, wherein the pathogen is a bacterial pathogen.
- 1                   44.     The method of claim 42, wherein the pathogen is a fungal pathogen.
- 1                   45.     The method of claim 42, wherein the pathogen is a viral pathogen.
- 1                   46.     The method of claim 45, wherein the viral pathogen is HIV.
- 1                   47.     The method of claim 46, wherein the biologically active protein is CD4  
2     or an HIV-binding fragment of CD4.
- 1                   48.     The method of claim 46, wherein the biologically active protein is 2D-  
2     CD4.
- 1                   49.     The method of claim 45, wherein the biologically active protein is  
2     cyanovirin-N or a virus-binding fragment of cyanovirin-N.
- 1                   50.     The method of claim 45, wherein the viral pathogen is herpes simplex  
2     virus.
- 1                   51.     The method of claim 45, wherein the biologically active protein is  
2     herpes simplex virus entry mediator C (HveC) or a virus-binding fragment of HveC.
- 1                   52.     The method of claim 27, wherein the biologically active polypeptide is  
2     released from the *Lactobacillus* bacterium.
- 1                   53.     The method of claim 30, wherein the biologically active polypeptide is  
2     anchored in the cell wall of the *Lactobacillus* bacterium.
- 1                   54.     A method of providing a biologically active protein to a mammalian  
2     mucosal surface, the method comprising,  
3                   contacting a mucosal surface with a *Lactobacillus* bacterium recombinantly  
4     altered to express a signal sequence linked to a biologically-active polypeptide linked to a  
5     heterologous carboxyl terminal cell wall targeting region, the heterologous carboxyl terminal  
6     cell wall targeting region comprising in the following order:

7 a cell wall associated sequence;  
8 LPQ(S/A/T)(G/A); and  
9 a hydrophobic sequence,  
10 wherein the biologically active polypeptide is expressed in an amount able to  
11 be detected in a sample collected from the mucosal surface.

1 55. The method of claim 54, wherein the cell wall associated sequence  
2 comprises at least 50 amino acids.

1 56. The method of claim 54, wherein the cell wall associated sequence  
2 comprises at least 200 amino acids.

1 57. The method of claim 54, wherein the heterologous carboxyl terminal  
2 cell wall targeting region further comprises a charged sequence at the carboxyl terminus of  
3 region.

1 58. The method of claim 54, wherein the *Lactobacillus* bacterium is  
2 selected from the group consisting of *L. jensenii*, *L. gasseri*, and *L. casei*.

1 59. The method of claim 54, wherein the mucosal surface resides within  
2 the vagina.

1 60. The method of claim 54, wherein the mucosal surface resides within  
2 the gastrointestinal tract.

1 61. The method of claim 54, wherein the contacting step comprises orally  
2 administering the *Lactobacillus* bacterium.

1 62. The method of claim 54, wherein the contacting step comprises  
2 vaginally administering the *Lactobacillus* bacterium.

1 63. The method of claim 54, wherein the contacting step comprises  
2 rectally administering the *Lactobacillus* bacterium.

1 64. An expression cassette comprising a promoter operably linked to a  
2 polynucleotide encoding a signal sequence and a biologically-active polypeptide, wherein the  
3 biologically active polypeptide is linked to SEQ ID NO:7 or SEQ ID NO:8.

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65. A vector comprising the expression cassette of claim 64.